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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: JOHN C. CHABALA et al

Group: 125

Serial No: 928,111

Case No: 16112IA

Examiner: B. Hazel

Filed: 7/31/78

For: SELECTIVE HYDROGENATION PRODUCTS OF C-076 COMPOUNDS AND DERIVATIVES

Commissioner of Patents & Trademarks
Washington, D. C. 20231

AFFIDAVIT UNDER 37 CFR 1.132

Sir:

I, Richard T. Robertson, being duly sworn, do hereby affirm and declare under oath:

THAT I am familiar with the chemical compounds identified as C-076 and the dihydro derivatives thereof;

THAT I am familiar with United States Patent Application Serial No. 928,111, which describes and claims compounds known as 22,23-dihydro C-076 compounds;

THAT I understand that the United States Patent and Trademark Office Examiner has rejected claims in the above-mentioned application over the patent application that discloses the parent C-076 compounds;

THAT I am a toxicologist, having received my Bachelor of Science degree in Chemistry from The Citadel, in Charleston, South Carolina in 1967, my Master of Science Degree in Biochemistry from the University of Georgia, in Athens, Georgia in 1969, and my Doctor of Philosophy in Biochemistry from the University of Georgia, in Athens, Georgia in 1971;

THAT I am currently employed as a toxicologist at the Merck Institute for Therapeutic Research at West Point Pennsylvania and have been so employed since April 1, 1975.

THAT part of my duties is to compile a single complete toxicological report containing individual reports on the safety and toxicological properties of certain of the compounds tested at the Merck Institute for Therapeutic Research. These reports are used to assess the safety and toxicity characteristics of compounds under development by Merck Sharp & Dohme Research Laboratories. Also these reports are submitted to the Food and Drug Administration in support of New Drug Applications and New Animal Drug Applications to demonstrate that the compounds for which approval is requested are adequately safe for introduction as a commercial drug product;

THAT in the case of the compounds C-076 Bla and of 22,23-dihydro C-076 Bla and Blb (the 80:20 mixture of which is referred to as MK933) such reports were prepared;

THAT before any such reports are issued, the data are audited for accuracy and completeness;

THAT attached hereto as Exhibit I is a copy of the summary of the toxicological report for 22,23-dihydro C-076 Bla and Blb consisting of 12 pages of text;

THAT attached hereto as Exhibit II is a copy of the table of contents of the complete report, which consists of six pages and indicates that the complete report consists of eight major sections and is in excess of 505 pages in total;

THAT attached hereto as Exhibit III is a copy of the summary of the toxicological report for C-076 Bla consisting of 14 pages of text;

THAT attached hereto as Exhibit IV is a copy of the table of contents of the complete report for C-076 Bla, which consists of three pages and indicates that the complete report consists of nine major sections and is in excess of 578 pages in total;

THAT I have examined the complete toxicological reports for C-076 Bla and 22,23-dihydro C-076 Bla and Blb and have prepared a comparison report in which the toxicological tests for each are compared on a test by test basis;

THAT the comparison report is as follows:

Introduction:

C-076 (B_{1a}) (L-676,895) and MK-933 (L-640,471) are macrocyclic lactone disaccarides with antiparasitic activity. The structure of MK-933 differs from that of C-076 (B_{1a}) in that the double bond between carbons 22 and 23 in C-076 (B_{1a}) has been reduced in MK-933. This structural modification has resulted in a substantial reduction in the toxicity of MK-933 compared to C-076 (B_{1a}). This decreased toxicity has been apparent in several species including mice, rats, and dogs under a variety of treatment regimens including single-dose acute oral administration, multiple-dose subacute oral toxicity studies, and teratology studies. The results of comparable toxicity studies conducted on both compounds are summarized in Table 1. These studies are discussed in detail in the individual toxicologic evaluations of the two compounds. Unless otherwise stated, the MK-933 tested was approximately an 80:20 mixture of 22,23-dihydro C-076 (B_{1a}) and 22,23-dihydro C-076 (B_{1b}). The C-076 (B_{1a}) used in these studies was from 95 to 99 percent pure.

Comparison of Results:

Neither C-076 (B_{1a}) or MK-933 was mutagenic in the Ames bacterial mutagen test.

The LD_{50} value (calculated single dose that will kill 50 percent of the test animals) of C-076 (B_{1a}) in adult female CF_1 mice is approximately one half to one third that of MK-933 at equivalent dosing concentrations (0.8 percent). The differences in acute oral toxicity for C-076 (B_{1a}) and MK-933 in male and female CRCD rats were even more pronounced. The LD_{50} of a 0.2 percent solution of C-076 (B_{1a}) in male and female rats was approximately one fourth to one fifth that observed with an 0.8 percent solution of MK-933. Since decreases in LD_{50} values with increasing concentration of a variety of test agents have been documented (Ferguson, H. C., Toxicology Appl. Pharmacol .4: 759-762, 1962), the differences in LD_{50} values between C-076 (B_{1a}) and MK-933 in rats may be even greater than the present data demonstrate. In spite of the large difference in toxicity between C-076 (B_{1a}) and MK-933 in adult rats, the LD_{50} values for C-076 (B_{1a}) in neonatal rats was only 35 percent less than that of MK-933.

Oral reproduction studies were conducted in which sexually mature female rats were administered either C-076 (B_{1a}) or MK-933 from 15 days prior to mating throughout gestation and lactation until Day 21 postpartum. The purpose of these studies was to produce weanling rats for use in 90-day oral toxicity studies that had been exposed to the test agent in utero and throughout lactation. The C-076 (B_{1a}) reproduction studies were conducted at doses of 0.1, 0.2, 0.4, 0.5, 1.0, and 1.5 mg/kg/day. The no-effect level in these studies was 0.1 mg/kg/day based on toxicity in neonatal rats. Toxic signs, including spastic movements and tremors, were observed in neonates at doses of C-076 (B_{1a}) as low as 0.2 mg/kg/day, and mortality resulted from doses equal to and greater than 0.5 mg/kg/day. The high mortality observed among neonates whose dams were dosed with 0.5, 1.0 and 1.5 mg/kg/day of C-076 (B_{1a}) precluded continuation of these dosage groups in a 90-day oral toxicity study. Weanling pups exposed to C-076 (B_{1a}) in utero at 0.1, 0.2, or 0.4 mg/kg/day were continued on a 90-day oral toxicity study and no mortality, physical signs of toxicity, or significant gross or histopathologic changes were noted.

The no-effect level in comparable MK-933 studies at doses of 0.4, 0.8, and 1.6 mg/kg/day was 0.4 mg/kg/day based on evidence of possible intravascular hemolysis observed in rats at the completion of the 90-day toxicity study. The only signs of toxicity observed among neonatal rats in this study was a very slight increase in mortality and some hypothermia at 1.6 mg/kg/day. There were no signs of toxicity in neonates at 0.4 or 0.8 mg/kg/day. In terms of toxicity observed in neonatal rats in these studies, C-076 (B_{1a}) is approximately eight times more toxic than MK-933. If toxicity is considered irrespective of age, than C-076 (B_{1a}) is at least four times more toxic than MK-933.

In an 18-week oral toxicity study in dogs in which C-076 (B_{1a}) was administered at 0.25, 0.5, 2.0 and 8.0 mg/kg/day, the no-effect level was 0.25 mg/kg/day. Death, tremors, ataxia, and mydriasis occurred at all other doses. In contrast, dogs administered MK-933 at doses of 0.5, 1.0, or 2.0 mg/kg/day for 14 weeks displayed similar toxic effects only at the 2.0 mg/kg/day dosage level. Mydriasis and a slight body weight loss were the only effects observed in the 1.0 mg/kg/day group, and the no-effect level in this study was 0.5 mg/kg/day. In addition, 4 of 8 dogs were able to survive 94 to 95 consecutive 2.0 mg/kg/day doses of MK-933, whereas in the C-076 (B_{1a}) study the 2.0 mg/kg/day dose had to be terminated after 3 doses when it became apparent that no dogs could be expected to survive at this dosage level. Based on the no-effect level in these studies, C-076 (B_{1a}) has two times the toxicity of MK-933 in dogs.

In a mouse teratology study in which C-076 (B_{1a}) was administered orally to pregnant mice from Days 6 to 15 of gestation at 0.1, 0.2, 0.4, and 0.8 mg/kg/day there were dose-related increases in maternal death at all dosage levels. Teratogenicity was apparent as an increase in the incidence of cleft palates in fetuses from dams given 0.4 or 0.8 mg/kg/day and the no-effect level based on teratogenicity

was 0.2 mg/kg/day. Since a no-effect level for the teratogenicity of C-076 (B_{1a}) had been established, a study was conducted to determine the no-effect level for maternotoxicity. C-076 (B_{1a}) was administered orally from Days 6 to 15 of gestation at doses of 0.025, 0.05, 0.075, and 0.10 mg/kg/day. The no-effect level for maternotoxicity in this study was 0.05 mg/kg/day. Deaths occurred at a dose of 0.1 mg/kg/day, and tremors and eventual coma were seen in one mouse at 0.075 mg/kg/day.

Mouse teratology studies were also conducted on the individual components of MK-933. The 22,23-dihydro C-076 (B_{1a}) component of MK-933 was administered orally to pregnant mice on Days 6 through 15 to gestation at dose levels of 0.2, 0.4, 0.8, and 1.6 mg/kg/day. The 22,23-dihydro C-076 (B_{1b}) component of MK-933 was administered at doses of 0.4, 0.8 and 1.6 mg/kg/day to pregnant mice in an identical treatment regimen. There was maternal mortality in mice administered 0.4, 0.8, and 1.6 mg/kg/day of 22,23-dihydro C-076 (B_{1a}), and teratogenicity as evidenced by cleft palates was seen in fetuses from dams treated with 1.6 mg/kg/day. Therefore, the no-effect levels for 22,23-dihydro C-076 (B_{1a}) were 0.8 mg/kg/day based on teratogenicity and 0.2 mg/kg/day based on maternotoxicity. Among pregnant mice administered 22,23-dihydro C-076 (B_{1b}) there was maternal mortality at 0.8 and 1.6 mg/kg/day, and teratogenicity, as evidenced by cleft palates, was apparent in fetuses from dams in the 0.8 and 1.6 mg/kg/day treatment groups. The no-effect level for both teratogenic and maternotoxic effects in this study was 0.4 mg/kg/day. Based on maternal mortality in these mouse teratology studies, C-076 (B_{1a}) is approximately four and eight times more toxic than the 22,23-dihydro C-076 (B_{1a}) and 22,23-dihydro C-076 (B_{1b}) components of MK-933, respectively. C-076 (B_{1a}) also had approximately four and two times the teratogenic potential in mice of the 22,23-dihydro C-076 (B_{1a}) and 22,23-dihydro C-076 (B_{1b}) components of MK-933, respectively.

Conclusions:

Both C-076 (B_{1a}) and MK-933 were highly toxic in all species and protocols evaluated. However, with the possible exception of a 35 percent decrease in LD_{50} values for infant rats, C-076 (B_{1a}) was at least two to four times more toxic in all test species and dosing regimens tested. In addition, the teratogenic potential of C-076 (B_{1a}) in mice was approximately two to four times greater than that of the individual components of MK-933.

THAT the foregoing comparison report was prepared from an examination of all of the data available on the compounds discussed in the complete toxicological report and that the data indicate that the 22,23-dihydro compounds are all less toxic than the parent C-076 compounds or in certain cases the compounds are the same. There are no data which demonstrate that the parent C-076 compounds are less toxic than the 22,23-dihydro compounds.

THAT while both the parent C-076 compounds and the 22,23-dihydro compounds have toxic properties in the animals and protocols evaluated above, the 22,23-dihydro compounds are significantly less toxic. This decrease in toxicity for the 22,23-dihydro compounds improves the safety of these compounds to humans, if present in edible tissue from animals of ultimate use which were treated with the 22,23-dihydro compounds, and makes these compounds commercially viable. The greater toxicity of the parent C-076 compounds results in a decrease in the safety of these compounds to humans, if present in edible tissues from animals of ultimate use which were treated with such parent C-076 compounds, and makes these compounds less usable commercially.

Further deponent sayeth not.

Richard T. Robertson

STATE OF NEW JERSEY

COUNTY OF UNION

Sworn to and subscribed before me this 13th day of July 1979.

Notary Public

NANCY ROMOND
NOTARY PUBLIC OF NEW JERSEY
My Commission Expires April 8, 1981